

Collaborative research project between International Livestock Research Institute (ILRI) and the Rural Development Administration (RDA)

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Introduction

The abiotic stresses drought and high temperature are the two major adverse effects of climate change that influence forage production. Thus, the identification of drought and heat stress tolerant forage cultivars is one of the key activities in forage development programs. Napier grass (*Cenchrus purpureus*) is an important forage crop in Eastern and Central Africa and across the global tropics. Napier grass grows from sea level in the tropics and produces best growth in temperatures ranging from 25–40°C (Cook et al. 2020). It is also considered a short-term drought tolerant forage, an important characteristic in areas such as Eastern and Central Africa which are affected by frequent drought conditions. Napier grass is popular among smallholder dairy farmers, especially in the cut and carry feeding system, as it produces high forage yield per unit area, can withstand repeated cuttings during a year and is mostly enjoyed by different livestock animals when compared with other tropical forages. To exploit the potential of Napier grass for improved water use efficiency (WUE), a field drought stress trial was conducted using accessions from both the collections of the International Livestock Research Institute (ILRI) and the Brazilian Agricultural Research Corporation (EMBRAPA). This was undertaken in Bishoftu, Ethiopia. Eighty-four accessions were evaluated for drought and heat stress tolerance during the dry seasons of 2018, 2019 and 2020 based on agro-morphological and feed nutritional quality performance under two soil moisture regimes—moderate (MWS) and severe (SWS) water stress—created by drip irrigation in the dry season and under rainfed conditions in the wet season (Wet). Overall, the results of the analysis of variance for agro-morphological and feed quality traits indicated the existence of significant phenotypic diversity among the experimental accessions. Consistent high biomass yielding accessions with enhanced WUE were observed across harvests in each soil moisture regime. In addition, a genome-wide association study (GWAS) was employed using a combination of the agro-morphological traits measured under the three soil moisture regimes and high-density genome-wide DArTseq markers. More than 58 quantitative trait loci (QTL) and markers associated with the important agro-morphological and water-use efficiency traits were identified.

Main results

The field experiment was conducted between 2017 and 2020 using the subset of 84 Napier grass accessions from the ILRI and EMBRAPA collections, planted in a partially replicated (p-rep) design with four replications. Cuttings of accessions were planted in June 2017. In the dry season (October–May), drip irrigation was used to supply water while in the wet season (June–September), plants used rainwater for growth. After the plants established well, drought stress was imposed in the dry season where two replications were exposed to moderate water stress (MWS) and the other two replications were treated with severe water stress (SWS) conditions that correspond to a volumetric soil water content of 20% and 10%, respectively. The soil water content of the blocks in the dry and wet seasons was monitored using a Delta soil moisture probe (HD, England) (Figure 1). In addition, a mini weather station (Spectrum technologies) was installed to monitor the daily climatic variables that were used to estimate evapotranspiration (Figure 2). Plants were harvested after every eight weeks of regrowth, resulting in a total of 12 harvests conducted between June 2018 and May 2020. In each harvest, morphological, agronomic, and feed quality traits were collected and analyzed for their forage performance value under different soil moisture conditions in the dry and wet seasons. The accessions were also genotyped using the DArTseq platform of Diversity Arrays Technology as described previously (Muktar et al. 2019). Marker-trait association analysis was carried out using the genotype and phenotype data in a mixed linear model.

Figure 1: Mean values of soil volumetric water content (VWC) percentage of blocks (1 and 2) treated with moderate water stress (MWS) and severe water stress (SWS) conditions across 12 harvests during the wet and dry seasons

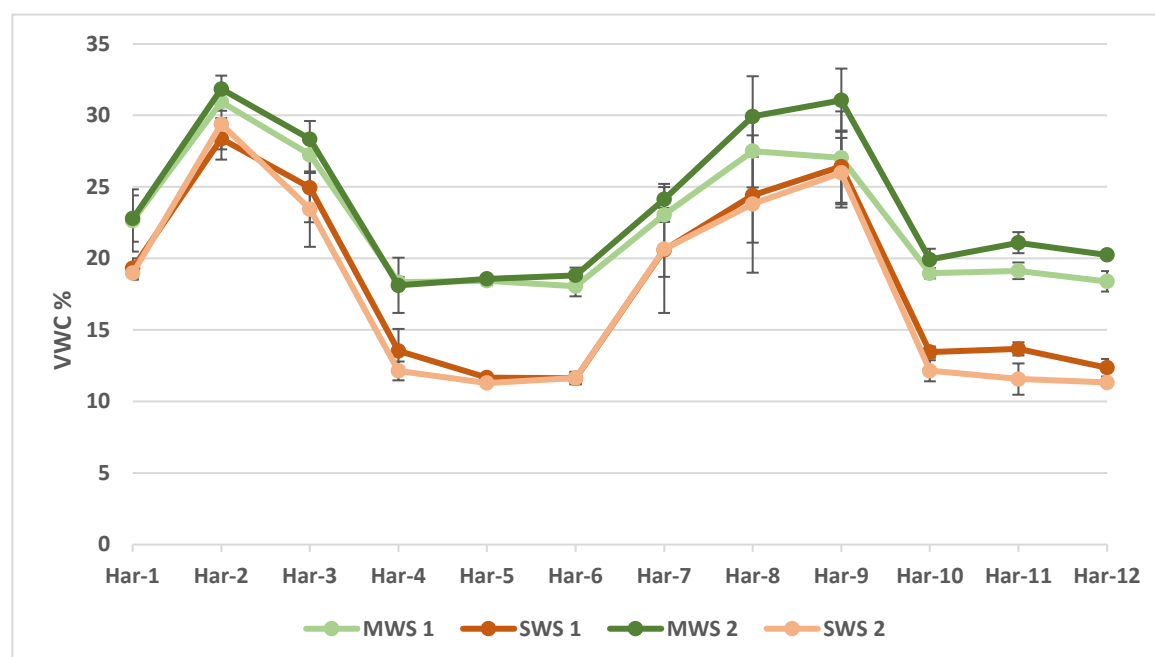
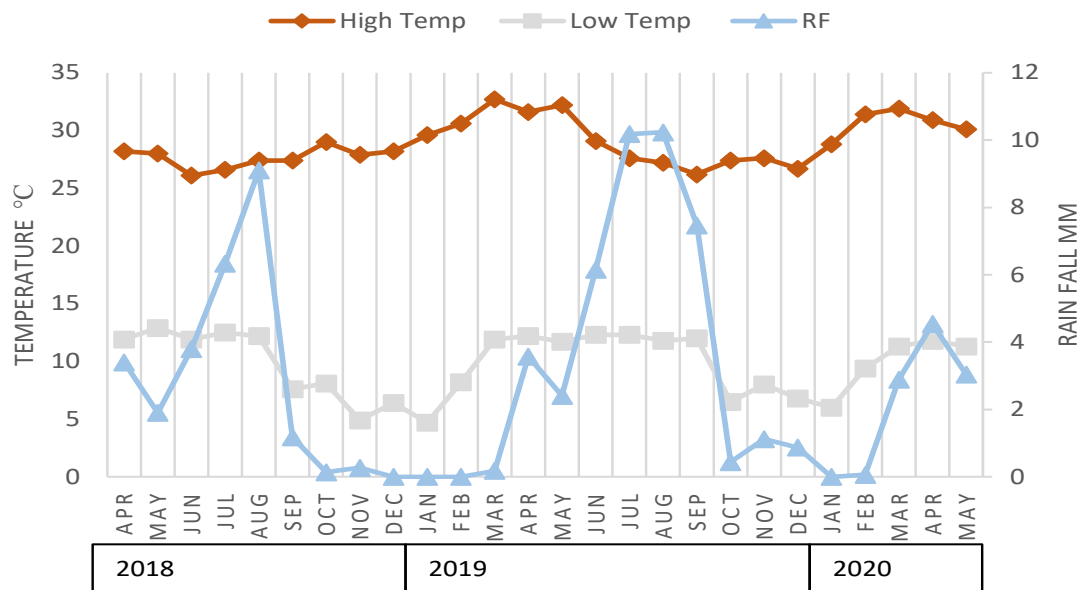


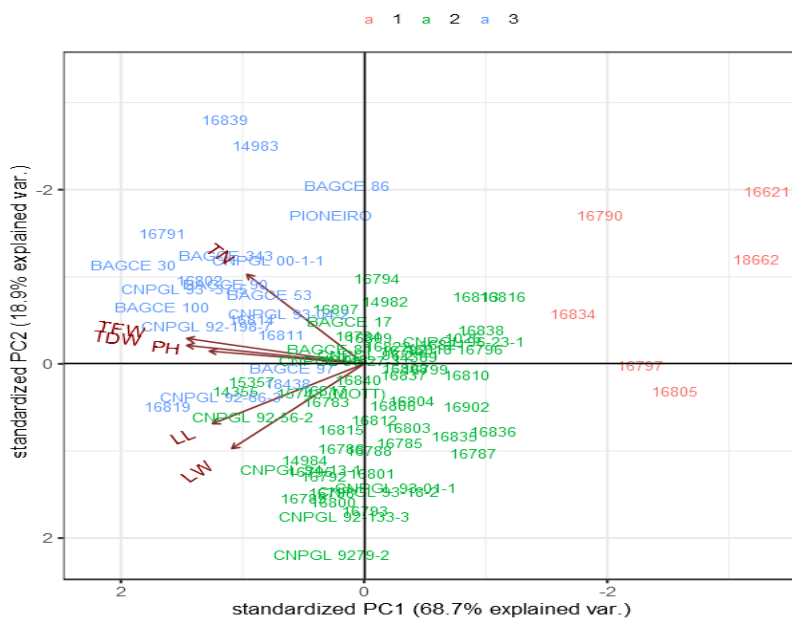
Figure 2: Monthly mean values of rainfall, high and low temperature variables of the Bishoftu field experimental site between 2018 and 2020



Forage yield performance

To evaluate the general performance of the 84 accessions, a cluster analysis was conducted by considering the two-year average data of the accessions for forage yield (total fresh weight and total dry weight) and yield related traits (plant height, tiller number, leaf length and leaf width). The result revealed three groups of accessions that can be delineated in terms of forage yield performance; these were broadly high, medium and low yielding groups (Figure 3).

Figure 3: Biplots of the first two principal components showing high (blue), medium (green) and low (red) yielding groups of the 84 Napier grass accessions based on yield related traits. Total fresh weight (TFW), total dry weight (TDW), plant height (PH), leaf length (LL), leaf width (LW) and tiller number (TN)



Napier grass accession performance under wet season conditions

Generally, the accessions showed significant genotypic variation for morphological, agronomic and feed quality traits (Tables 1 and 2). These phenotypic performance variations between accessions are indicative of the opportunity to select accessions for increased performance under optimum soil moisture conditions. However, there was no statistically significant difference for biomass traits during the wet season, between the blocks that were treated with different soil moisture levels—MWS and SWS—in the dry season. This finding indicates that the plants grown under the SWS treatment recovered more strongly with the onset of the wet season rains. Thus, the plants showed a higher ability to recover and continue to grow under higher soil moisture conditions in the wet season (Figure 1). This recovery potential is an important response to determine the overall productivity performance of the accessions. Top potential candidates were identified for total dry weight (TDW) production that could be used under optimum water production conditions (Table 5). Furthermore, the crude protein yield (CPY) of these productive accessions were also the highest among the accessions (Table 6).

Table 1. Summary ANOVA for morphological and agronomic traits during wet season harvests

Sources of variation	Traits (P-Level (5%))										
	PH	LW	LL	IL	ST	TN	Fv/Fm	PI	TFW	TDW	LSR
Genotypes	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
Treatments (MWS/SWS)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Harvest	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
Genotype X treatment	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype X harvest	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
Treatment X harvest	NS	NS	< .001	< .001	NS	< .001	< .001	< .001	< .001	< .001	< .001
Genotype X treatment X harvest	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV %	2.9	1.6	2	49.8	2.2	12.3	5.6	9.9	6.2	9.4	54.6

Plant height (PH), leaf width (LW), leaf length (LL), internode length (IL), stem thickness (ST), tiller number (TN), photosynthesis efficiency (Fv/Fm), performance index (PI), total fresh weight (TFW), total dry weight (TDW) and leaf to stem ratio (LSR).

Table 2. Summary ANOVA for forage nutrition quality parameters from leaf and stem samples during wet season harvests

Sources of variation	Traits (P-Level (5%))									
	NDF		OM		CP		IVOMD		ME	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Genotypes	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
Treatments (MWS/SWS)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Harvest	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
Genotype X treatment	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype X harvest	NS	NS	0.001	0.001	NS	< .001	NS	< .001	NS	< .001
Treatment X harvest	< .001	0.009	< .001	0.001	NS	< .001	< .001	< .001	< .001	< .001
Genotype X treatment X harvest	NS	0.06	NS	0.041	NS	NS	NS	0.03	NS	0.03
CV %	3.5	5.9	1.3	1.6	15.5	25.6	3.3	5.2	3.2	4.9

Neutral detergent fibre (NDF), organic matter (OM), crude protein (CP), *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME).

Napier grass accession performance under dry season conditions

The results from the dry season harvests revealed significant genotype, treatment and harvest effects for morpho-agronomic traits (Table 3). Genotype by treatment interaction also revealed significant differences, indicating the performance of individual accessions differ between MWS and SWS treatments. The performance of the accessions was affected differentially by the harvest. This could be related to variations in the existing climatic variables in each eight-week regrowth period (Figure 2).

Overall, these genotypic variations are important to exploit the potential of the accessions to maximize forage production under different water stress environments. Thus, the top biomass yielding accessions identified based on total dry weight (TDW) would be potential candidates for future utilization and breeding programs for drought stress environments (Table 5).

The accessions also showed significant genotypic variation for feed quality traits; no differences in feed quality traits were detected between the treatment blocks while the difference between individual harvests was significant (Table 4). In terms of crude protein yield (CPY), top producing accessions were generally similar in the MWS and SWS conditions, indicating the possibility of the development of high feed quality Napier grass accessions via selection for drought stress environments (Table 6).

Table 3. Summary ANOVA for morphological and molecular traits from moderate and severe water stress conditions in dry season harvests

Sources of variation	Traits (P-Level (5%))								
	PH	LW	LL	TN	Fv/Fm	PI	TFW	TDW	WUE
Genotypes	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
Treatments (MWS/SWS)	0.05	0.04	0.03	< .001	0.04	0.07	< .001	0.05	0.05
Harvest	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
Genotype X treatment	0.02	NS	< .001	< .001	NS	< .001	< .001	< .001	< .001
Genotype X harvest	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001
Treatment X harvest	< .001	< .001	< .001	< .001	< .001	< .001	< .001	< .001	NS
Genotype X treatment X harvest	< .001	NS	< .001	< .001	NS	< .001	< .001	< .001	< .001
CV %	11.2	9.4	6.1	5.2	2.6	20.7	13.3	17.8	13.4

Plant height (PH), leaf width (LW), leaf length (LL), tiller number (TN), photosynthesis efficiency (Fv/Fm), performance index (PI), total fresh weight (TFW), total dry weight (TDW) and water use efficiency (WUE)

Table 4. Summary ANOVA for forage nutrition quality parameters from leaf and stem samples from moderate and severe water stress conditions in dry season harvests

Sources of variation	Traits (P-Level (5%))				
	NDF	OM	CP	IVOMD	ME
Genotypes	< .001	< .001	< .001	< .001	< .001
Treatments (MWS/SWS)	0.024	NS	NS	NS	NS
Harvest	< .001	0.001	< .001	< .001	< .001
Genotype X treatment	< .002	NS	NS	NS	NS
Genotype X harvest	0.026	NS	< .001	< .001	< .001
Treatment X harvest	< .001	NS	NS	NS	NS
Genotype X treatment X harvest	NS	NS	NS	NS	NS
CV %	7.1	3.1	27.1	5.5	5.3

Neutral detergent fibre (NDF), organic matter (OM), crude protein (CP), *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME).

Table 5. Mean total dry weight (TDW) of top yielding Napier grass accessions per harvest in the wet season (Wet), moderate water stress (MWS) and severe water stress (SWS). Superscripted letters indicate performance differences at the 5% significance level.

Accession #	Wet-TDW (t/ha)	Accession #	MWS-TDW (t/ha)	Accession #	SWS-TDW (t/ha)
16791	20.4 ^a	16819	2.8 ^a	16819	2.5 ^a
16819	18.3 ^b	16803	2.7 ^{ab}	CNPGL 92-66-3	2.4 ^{abc}
BAGCE 30	17.4 ^c	16839	2.5 ^{bc}	16839	2.3 ^{bcd}
CNPGL 93-37-5	16.4 ^d	BAGCE 30	2.5 ^{bcd}	BAGCE 100	2.3 ^{bcd}
16802	16.3 ^d	16811	2.5 ^{bcd}	BAGCE 30	2.2 ^{bcd}
BAGCE 100	16.2 ^d	16795	2.4 ^{cde}	16791	2.2 ^{cd}
BAGCE 34	15.4 ^e	CNPGL 92-66-3	2.4 ^{cde}	16795	2.2 ^{cd}
BAGCE 93	15 ^{ef}	CNPGL 93-37-5	2.4 ^{cde}	CNPGL 93-37-5	2.2 ^{cd}
CNPGL 92-198-7	14.9 ^{ef}	BAGCE 93	2.3 ^{def}	16802	2.2 ^{cd}
15357	14.7 ^f	14982	2.3 ^{ef}	BAGCE 93	2.1 ^d

Table 6. Mean crude protein yield (CPY) of top Napier grass accessions per harvest in in the wet season (Wet), moderate water stress (MWS) and severe water stress (SWS). Superscripted letters indicate performance differences at the 5% significance level.

Accession #	Wet-CPY (t/ha)	Accession #	MWS-CPY (t/ha)	Accession #	SWS-CPY (t/ha)
16791	215 ^a	16811	37.07 ^a	BAGCE 93	48.78 ^a
16819	206.8 ^{ab}	BAGCE 93	28.68 ^b	16811	44.65 ^{ab}
BAGCE 30	195.1 ^{bc}	16839	28.07 ^{bc}	16819	44.25 ^{ab}
16802	183.2 ^{cd}	16819	25.67 ^{bcd}	16839	41.61 ^{ab}
CNPGL 93-37-5	173.2 ^{de}	CNPGL 00-1-1	25.25 ^{bcd}	CNPGL 92-66-3	39.86 ^{ab}
CNPGL 00-1-1	164.4 ^{ef}	BAGCE 30	25.09 ^{bcd}	CNPGL 92-56-2	39.31 ^{ab}
CNPGL 92-198-7	163.5 ^{ef}	CNPGL 92-56-2	24.09 ^{bcd}	BAGCE 30	38.19 ^{ab}
BAGCE 34	161.4 ^{ef}	16795	23.93 ^{bcd}	CNPGL 92-198-7	37.76 ^{bc}
BAGCE 93	160 ^{ef}	16803	23.24 ^{bcd}	16795	37.01 ^{bc}
BAGCE 100	156.3 ^f	BAGCE 97	22.7 ^{bcd}	16803	36.35 ^{bc}

High density genome-wide DArTseq markers and their genomic position on the Napier grass genome

The 84 accessions were genotyped using the genotyping-by-sequencing method of the DArTseq platform, which produced more than 200,000 high density genome-wide SilicoDArT and SNP markers (Muktar et al. 2019). A total of 135,706 silicoDArT and SNP markers were filtered after removing markers with missing values > 10% and a minor allele frequency (MAF) < 5% and used in a GWAS. The markers were mapped on to the recently reported Napier grass genome (Yan et al. 2020) and approximately 80% of the markers were able to be mapped on the fourteen chromosomes (LG, linkage groups).

QTL regions and markers associated with the important agro-morphological and water use efficiency traits

A genome-wide association study (GWAS) using mixed linear models identified more than 58 QTL regions and markers associated with the important agro-morphological and water use efficiency traits under the three-soil moisture conditions (Wet, MWS and SWS) (Figures 5 and 6).

Figure 4: Manhattan plots showing marker associations with agronomic and morphological traits in the wet season (Wet)

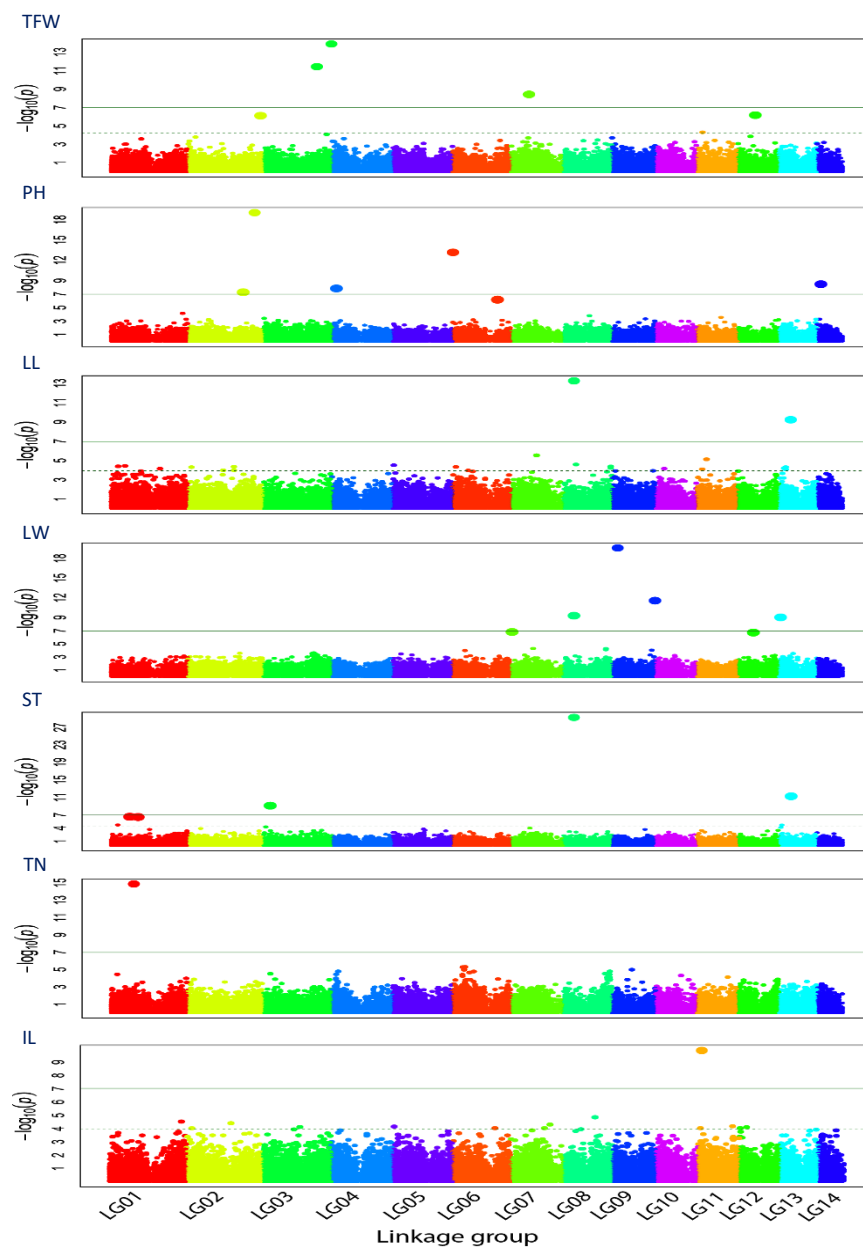


Figure 5: Manhattan plots showing marker associations with agronomic and morphological traits in the dry season under moderate water stress (DS-MWS)

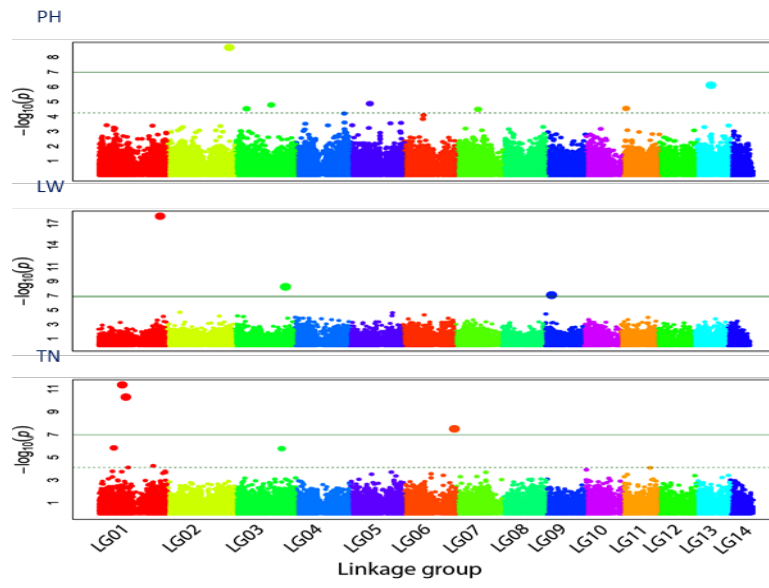
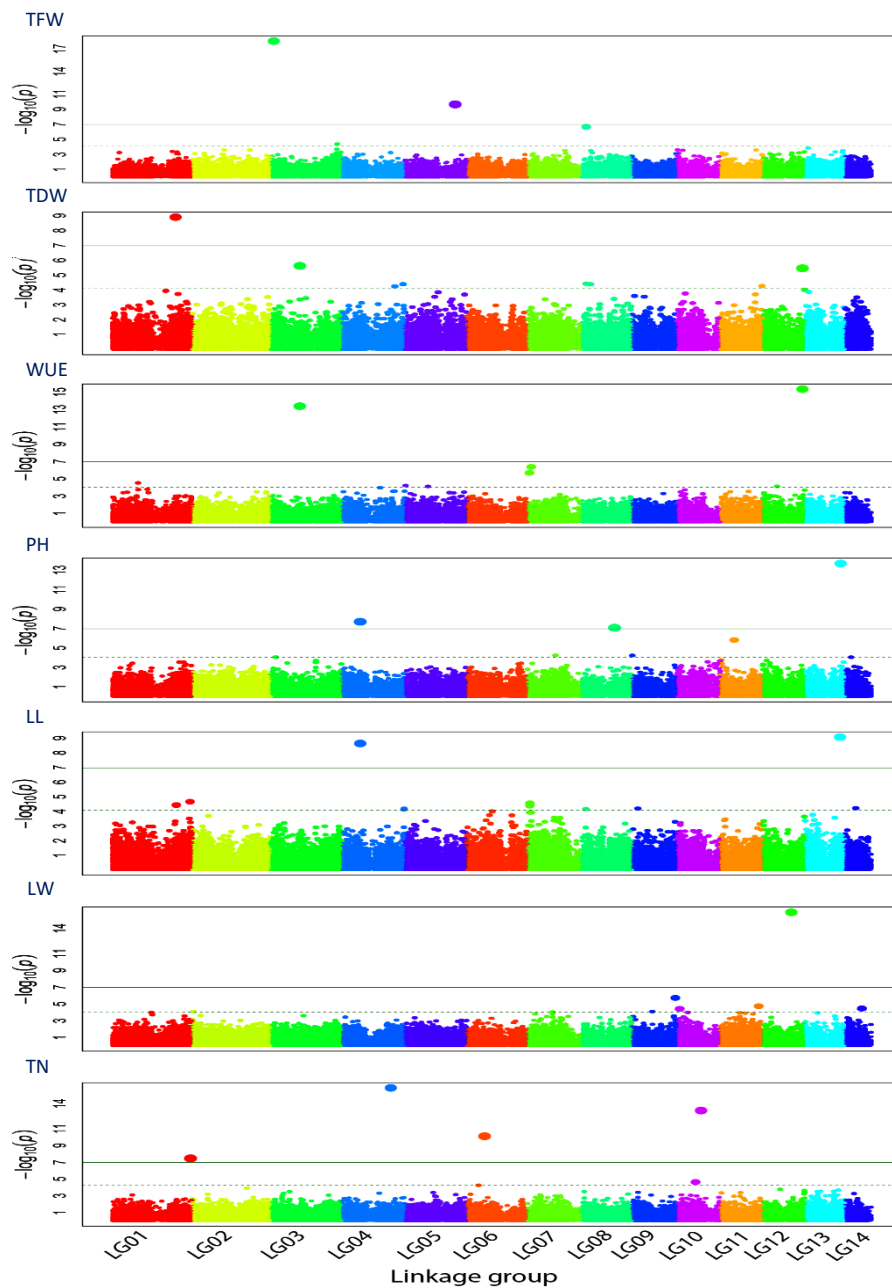


Figure 6: Manhattan plots showing marker associations with agronomic and morphological traits in the dry season under severe water stress (DS-SWS)



A total of 28 markers associated with the TFW, PH, LL, LW, TN, ST and IL traits were identified under the Wet condition. The 28 markers were distributed across most chromosomes, except for LG04, LG05 and LG10. Under the MWS condition, eight markers showed an association (Figure 5). The eight markers were associated with the PH, LW and TN traits and were distributed across LG01, LG02, LG06, LG09 and LG13. Three markers on LG01 showed the strongest association with the LW ($P < 9.52E-19$) and TN ($P < 5.15E-11$) traits. A total of 22 markers showed an association with the TFW, TDW, WUE, PH, LL, LW and TN traits under the SWS condition (Figure 6). The markers were distributed across most chromosomes, except for LG02, LG09 and LG14. Two markers on LG03 and

LG12 showed an association with the two most important agronomic traits—TDW and WUE. The strongest association with WUE was observed for two markers on LG03 ($P < 4.15E-14$) and LG12 ($P < 4.44E-16$). Another marker on LG03 showed the strongest association ($P < 9.46E-19$) with the TFW trait (Figure 6). A total of eleven markers showed an association with the morphological traits (PH, LL, LW and TN), of which the strongest association was observed for two markers on LG13 with the PH and LL traits; one marker on LG12 with the LW trait; and another three markers on LG04, LG06, LG10 with the TN trait. One marker on LG04 showed an association with both PH and LL traits (Figure 6).

Seventeen markers associated with TN, LL, PH, LW and ST traits were detected as common markers across the three soil moisture conditions (Wet, MWS and SWS). Of these, three markers on LG01, LG07 and an unmapped position were associated with the TN trait in all the three soil moisture conditions. Between Wet and MWS conditions, eight common markers on LG02, LG03, LG08, LG11 and LG13, associated with the PH, LL, LW, TN and ST traits, were detected. Five common markers on LG02, LG09 and an unmapped position associated with the LL and TN traits were detected in both the Wet and SWS conditions. Only one common marker, which was on LG04 and associated with the PH trait, was detected in both the MWS and SWS conditions.

The markers identified in this study can be used in marker-assisted selection (MAS) to develop high biomass producing Napier grass varieties with enhanced water use efficiency for dry and water deficit conditions. Furthermore, the associated markers and candidate genes aligned with them will be useful for further characterization and cloning of the QTLs.

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